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Activation of membrane progesterone receptor-alpha increases proliferation, migration, and invasion of human glioblastoma cells

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ABSTRACT

Background and aims: Glioblastoma is the most frequent and aggressive brain tumor due to its high capacity to migrate and invade normal brain tissue. The steroid hormone progesterone (P4) contributes to the progression of glioblastoma by promoting proliferation, migration, and cellular invasion through the activation of its intracellular receptor (PR). However, the use of PR antagonist RU486 partially blocks the effects of P4, suggesting the participation of signaling pathways such as those mediated by membrane receptors to P4 (mPRs). Therefore, this study aimed to investigate the effects of mPRα subtype activation on proliferation, migration, and invasion of human glioblastoma cells.

Methods: We treated human glioblastoma cell lines U87 and U251 with the specific mPRα agonist Org OD 02-0, and evaluated its effects on cell number, proliferation, migration, and invasion. Additionally, we measured the phosphorylation of the kinases Src and Akt in both cell lines upon Org OD 02-0 treatment.

Results: Org OD 02-0 (100 nM) augmented the number of U87 and U251 cells by increasing cell proliferation. The treatment with this agonist also increased U87 and U251 cell migration and invasion. Both proliferation and cell invasion decreased when mPRα expression was silenced. Finally, we observed that Org OD 02-0 increased the content of p-Src and p-Akt in both cell lines.

Conclusion: Our data suggest that P4 produces its effects in human glioblastoma progression not only by PR interaction but also through cell signaling pathways activated by mPRα.

1. Introduction

Glioblastoma, a grade IV astrocytoma, is the most aggressive brain tumor in humans that arises from uncontrolled proliferation of glial and precursor glial cells (Louis et al., 2016). Glioblastoma occurs at all ages with a remarkable frequency between 40 and 70 years-old (Zhang et al., 2017; Stupp et al., 2005). The median survival is 12–16 months after diagnosis, giving glioblastomas the highest rate of death by brain tumors (Rich et al., 2005; Furnari et al., 2007; Nachbichler et al., 2017).

Progesterone (P4) is a steroid hormone mainly synthesized in ovaries and placenta (Graham and Clarke, 1997), but neurons and glial cells at specific regions of the central nervous system (CNS) produce P4, referred then as a neurosteroid. In the brain, P4 regulates physiological functions related to sexual behavior, neuroprotection, myelination, and learning and memory (Schumacher et al., 2012; Rossetti et al., 2016; Colciago et al., 2015). In addition to these effects, it contributes to glioblastoma progression, since there is experimental evidence of P4 promoting proliferation, migration, and infiltration of human-derived glioblastoma cells *in vitro* and *in vivo* models (González-Agüero et al., 2007; Germán-Castelán et al., 2014; Piña-Medina et al., 2016).

P4 exerts its multiple physiological effects by two central pathways: the classical and the non-classical. Through the classical pathway, P4 binds to its intracellular receptor (PR), a transcription factor that once active binds to specific DNA sequences called P4 response elements located in gene promoter regions, thus regulating their expression (Liu and Ogle, 2002; Camacho-Arroyo et al., 2002). Meanwhile, the non-classical pathway involves several mechanisms related to rapid cellular responses, including the PR ligand-independent activation by

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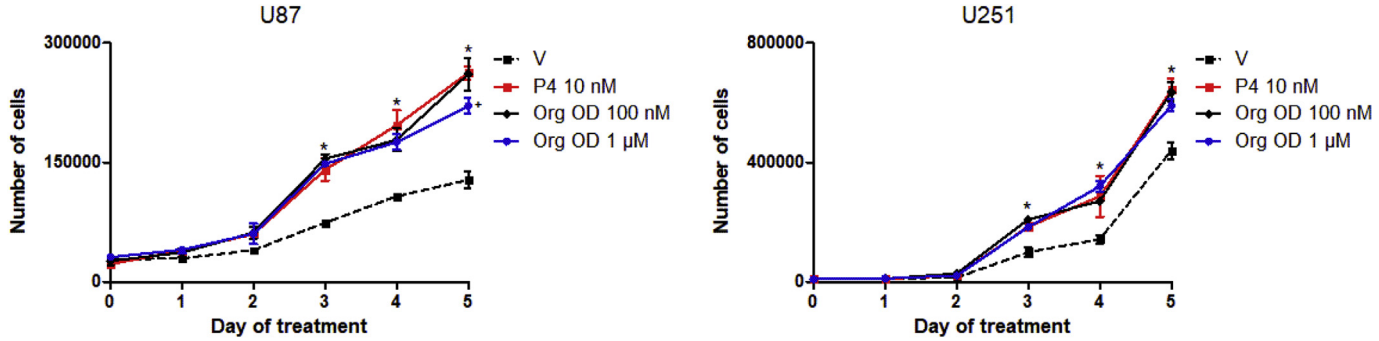

Fig. 1. P4 and mPRα agonist increase the number of U87 and U251 cells. Cells were treated with mPRα agonist Org OD 02-0 (100 nM and 1 μM), P4 (10 nM), and vehicle (V, 0.1% DMSO) for five days and the number of cells was determined each day by using the trypan blue dye exclusion assay. The results are expressed as the mean ± S.E.M.; *p < 0.001 *vs* V; + p < 0.05 *vs* P4.

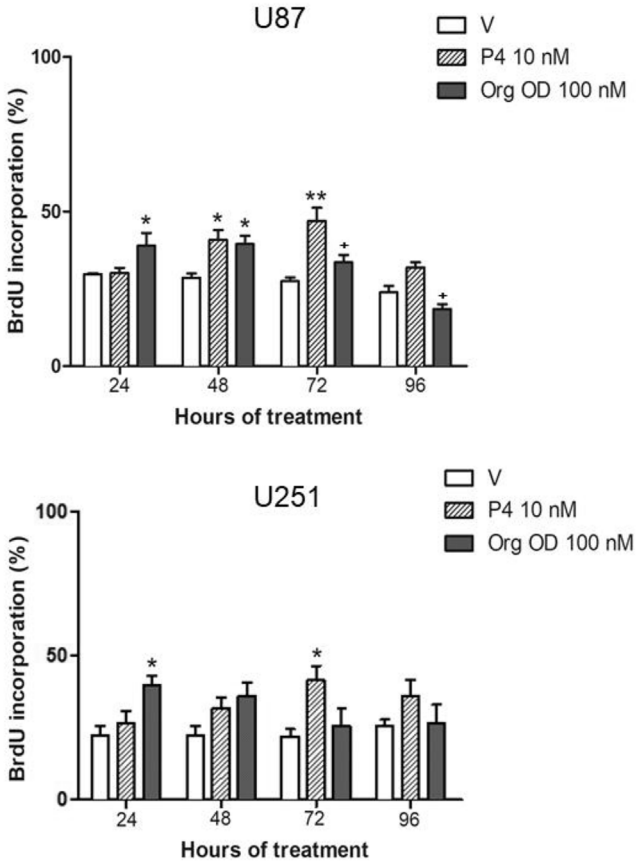

Fig. 2. P4 and the mPRα agonist Org OD 02-0 increase U87 and U251 cell proliferation. A BrdU incorporation assay was performed in cells treated with Org OD 02-0 (100 nM), P4 (10 nM), and vehicle (V, 0.1% DMSO) for 24–96 h. The number of BrdU positive cells was determined as a percentage of the total number of cells stained with the nuclei dye Hoechst. The data represent the mean ± S.E.M.; *p < 0.05; **p < 0.01 *vs.* V and +p < 0.05 *vs.* P4.

membrane-associated kinases, and the activation of different G protein-coupled membrane receptors to P4 (mPRs) (Boonyaratanakornkit et al., 2008; Zhu et al., 2003a; Valadez-Cosmes et al., 2016).

The human-derived glioblastoma cell lines U87, U251, and D54 express PR and their growth rate increases as a response to P4 (González-Agüero et al., 2007; Khalid et al., 1997; Germán-Castelán et al., 2016; Marquina-Sánchez et al., 2017). However, the use of mifepristone (RU486), a PR antagonist, partially inhibits P4 effects on proliferation and infiltration of glioblastoma cells (González-Agüero et al., 2007; Germán-Castelán et al., 2014; González-Arenas et al., 2014). In fact, mifepristone can exert agonist activity on PR as reported (Piña-Medina et al., 2016). This partial inhibition suggests the participation of other non-classical signaling pathways such as those activated by mPRs.

mPRs are G protein-coupled receptors that belong to the Progestin and AdipoQ Receptor Family (PAQR) (Zhu et al., 2003b), and five subtypes have been described: mPRα (PAQR7), mPRβ (PAQR8), mPRγ (PAQR5), mPRδ (PAQR6), and mPRε (PAQR9) (Valadez-Cosmes et al., 2016; Pang et al., 2013; Tang et al., 2005). The distribution of mPRs is tissue-specific, although different regions of the CNS show a high expression of mPRα, mPRβ, mPRδ, and mPRε. Notably, both neuronal and glial cells express mPRα, whose expression has been detected in the cortex, striatum, thalamus, hypothalamus, hippocampus, and cerebellum (Valadez-Cosmes et al., 2016; Meffre et al., 2013). Functionally, it has been proposed that mPRα, mPRβ, and mPRγ are coupled to inhibitory G proteins while mPRδ and mPRε are coupled to stimulatory G proteins (Zhu et al., 2003a, 2003b; Thomas, 2008; Thomas et al., 2007). Activation of mPRs triggers intracellular signaling cascades related to the modulation of cAMP levels, mobilization of intracellular Ca^2+, or regulation of ion channels that lead to the activation of specific kinases such as PI3K/Akt, c-Src, or MAPK (Zhu et al., 2003a; Valadez-Cosmes et al., 2016).

mPRs regulate many reproductive functions in P4 target tissues such as sexual behavior, maintenance of pregnancy, mammary gland development, and ovulation (Fernandes et al., 2005; Macias and Hinck, 2012; Mesiano et al., 2011). Nonetheless, their participation in ovarian and breast cancer development has also been demonstrated (Dressing and Thomas, 2007; Zuo et al., 2010; Charles et al., 2010; Pang and Thomas, 2011; Xie et al., 2012). Regarding glioblastomas, our laboratory has recently reported mPRα and mPRβ expression in the human-derived glioblastoma cell lines U87 and U251 (Valadez-Cosmes et al., 2015), however, it is still unknown if these cells respond to P4 through the activation of mPRs.

In the present study, we investigated the possible participation of mPRα in glioblastoma cell proliferation, migration, and invasion by using the specific agonist 10-ethenyl-19-norprogesterone (Org OD 02-0). The use of this agonist represents a reliable strategy to differentiate between the effects induced by mPRα or PR since experimental evidence shows that Org OD-02 0 neither binds nor exerts agonist effects on PR, while it binds to mPRα with a higher affinity than P4 (Kelder et al., 2010). Our results demonstrate that Org OD 02-0 increases cell proliferation, as well as the migration and invasion capacity of U87 and U251 cells. Silencing mPRα expression diminished U87 cell proliferation and invasion. Org OD 02-0 increased the content of p-Src and p-Akt, kinases that are related to signaling pathways induced by mPRs (Fu et al., 2010; Xie et al., 2013). These data suggest that P4 should mediate its effects on glioblastomas progression through cell signaling pathways activated by mPRα.